Specification

Carbohydrate absorption inhibitor and method for manufacturing the same

FIELD OF THE INVENTION

This invention relates to carbohydrate absorption inhibitor and method for manufacturing the same effective against e.g. diabetes prevention, diet, and so forth.

BACKGROUND OF THE INVENTION

Polysaccharide (starch, glycogen or the like) contained in food is hydrolyzed by α-amylase in saliva and pancreas and converted into oligosaccharide such as maltose, isomaltose or the like, then decomposed to glucose by disaccharide degrading enzyme such as α-glucosidase or the like in the small intestine and absorbed.

Thus, α-amylase and α-glucosidase perform an important role as a digestive enzyme for carbohydrate in the body. These enzyme activities affect the blood glucose level. Therefore, it is important to control these enzyme activities for the preventions and medical treatments of diabetes mellitus and obesity.

There are two different types of diabetes mellitus. One is insulin-dependent diabetes mellitus (Type I diabetes) and the other is non-insulin dependent diabetes mellitus (Type II diabetes), and the latter type accounts for more than 90% in the incidence of diabetes mellitus.

The latter type (Type II diabetes) is caused by derangement of carbohydrate and fat metabolism arising from an insufficient insulin action (for instance, insulin-hyposensitivity in the organ, insulin-hyposecretion in pancreas or the like) due to overeating.

Non-insulin dependent diabetes mellitus (Type II diabetes) should be basically controlled by diet therapy or exercise therapy rather than medication. However, such self-control does not always correct the aforementioned metabolic derangement, and medication is very much counted on.

However, it is necessary to consider side effects, dosage and usage of the medicine. In general, medicine is usually an intermixture of various components. Therefore, it is necessary to consider the side effect each component has and adverse effect caused by a prolonged dosage of each component.

Extract from gymnema leaf, guava leaf, banaba leaf, mulberry leaf or the like is widely known as a plant-derived extract which is effective to correct the derangement of carbohydrate metabolism. From the viewpoint of safety, these plant-derived extracts are easily applied to food, drugs and medicines, or the like, therefore effective components or the like of the said plant-derived extracts are now being extensively researched.

Against this background, the inventors in this invention have been investigating contents of polyphenol, SOD-like activities or the like in regard to the various plant-derived extracts, then finally came to focus on ethanol extract of evening primrose seed.

After each of the aforementioned experiments has been conducted, they came to know the fact that this extract contains various polyphenol effective to inhibit the carbohydrate absorption.

This invention aims at providing inhibitor of carbohydrate absorption derived from evening primrose seed and method for manufacturing the said inhibitor of carbohydrate absorption which are significantly effective to prevent or relieve symptoms of diabetes mellitus and to prevent obesity.

SUMMARY OF THE INVENTION

Carbohydrate absorption inhibitor which is the subject matter in this invention is characterized by comprising an effective component of solvent extract of compressed cake derived from the defatted evening primrose, and wherein the said effective component contains polyphenol such as ellagic acid, gallic acid, pentagalloylglucose.

Carbohydrate absorption inhibitor in this invention is characterized in that extracting solvent of the compressed cake derived from the aforementioned evening primrose seed is aqueous ethanol (water / ethanol = 15 to 30 / 70 to 85% (v/v)).

Carbohydrate absorption inhibitor in this invention is characterized in that defatting process is proceeded by using fat-soluble organic solvent.

Chemicals in this invention are characterized in that the aforementioned carbohydrate absorption inhibitor is contained.

Food and drink in this invention are characterized in that the aforementioned carbohydrate absorption inhibitor is contained.

According to the experiment conducted by the inventors, the existing plant-derived extracts (guava, banaba and mulberry) have a strong inhibitory action against amylase, however the enzyme inhibitory activity against amylase is not sufficient.

On the contrary, extract (polyphenol) derived from evening primrose as disclosed in this invention indicates strong enzyme inhibition activities against amylase and glucosidase. Especially the ethanol extract derived from the defatted evening primrose seed comprises a strong inhibitory action and consistent safety. Therefore it is appropriate to mix it with medicine to prevent diabetes mellitus or to diet

Evening primrose is an annual grass, biennial grass or herbaceous perennial of oenothera genus. In rare cases, stem of evening primrose may be lignified and grow on bush. The followings are four representative examples.

① Oenothera laciniata

② Oenothera striata

③ Oenothera biennis

④ Oenothera erythrosepala

In this invention, species of evening primrose are not limited. Any species can be used.

Oil obtained from evening primrose seed is widely known. The evening primrose oil contains a large amount of γ-linolenic acid and is said to be

effective against obesity, diabetes mellitus, hypercholesterolemia, alcohol dependence, aging, and disease caused by insufficient vitamin B_6.

Also it is widely known that evening primrose oil is effective to inhibit the action in which linolic acid is converted into γ-linolenic acid when virus infection is experienced, as well as to provide a medical treatment for asthma and atopic disease.

In regard to the fat-soluble component of evening primrose seed, physiological activity is now being researched, however, there is no report about the physiological activity of other substance than fat-soluble component.

Some part of the compressed cake exhausted in the manufacturing process of evening primrose seed is used as an animal feeding staff, or is disposed of as an industrial waste.

In this invention, a new usage of other components than the fat-soluble component of the evening primrose seed can be provided, and at the same time the effective component can be extracted from the compressed cake after taking out the evening primrose oil. It is significant to extract the high value-added effective component from the compressed cake and efficiently recycle the natural resources.

To efficiently extract the effective component (polyphenol) for the carbohydrate absorption inhibition, alcohol is used as an extracting solvent for the aforementioned evening primrose seed. Ethanol is especially recommended. If ehanol is used, effective component (polyphenol) is efficiently extracted and

can be used both for external application and food. According to other usages available, methanol, butanol or the like can be used.

The concentration of ethanol as an extracting solvent is preferably 70 to 85% (v/v). If the concentration of ethanol is less than 70% (v/V), polyphenol is not sufficiently extracted. And if the concentration exceeds 85% (v/v), seed oil easily dissolves into the extracting solvent.

Also, alcohol extraction should be repeated preferably at different levels of concentration since the content ratio of polyphenol is improved.

Furthermore, it is preferable to use the defatted evening primrose seed for alcohol extraction since polyphenol is concentrated in the defatted matter by removing oil from the seed. For instance, it is preferable to separate the oil from the evening primrose seed by compressing the said seed, and then to extract and separate the remaining oil of the compressed matter by fat-soluble organic solvent.

It is preferable to use hexane as a fat-soluble organic solvent for defatting since the extracted oil can be used for edible oil and also extract of defatted evening primrose seed can be used for foodstuff or the like. If the extract is used for other objectives than food, other non-polar solvent than hexane can be also used.

The aforementioned polyphenol derived from the evening primrose seed can be extracted preferably by solvent such as ethanol, aqueous ethanol, water or the like. According to the research by inventors, the extract contains gallic acid, ellagic acid, catechin, pentagalloylglucose.

Medicine described in this invention is suitable for the prevention of diabetes mellitus, i.e. the prevention or treatment of non-insulin dependent diabetes mellitus (Type II diabetes), or can be also used for the effective component of antiobesity drugs. For instance, medicine in this invention can be orally administered in any shape of soft and/or hard capsule, tablet, granule, fine granule, or powder medicine. On the other hand, the medicine in this invention can be administered by injection into body tissue, dermis, subdermal, muscle or vein,

Given dose varies depending on the mode of administration, condition of the disease, age of the patient, or the like. Adults can take the effective component of 0.5 to 5,000 mg per day and children can take 0.5 to 3,000mg per day.

Concentration of polyphenol component can be varied according to various types of medicine. However, when the medicine is used by oral administration or mucomembranous absorption, the given dose should be 0.01 to 15.0wt%. When the medicine is used by non-oral administration, the given dose should be 0.01 to 10wt. The dose mentioned above is only an example and can be varied according to various situations.

This invention suggests various embodiments of ordinary food and drink including snack food (such as chewing gums, candies, caramel candies (taffies), chocolates, cookies, jellies, gummy candies, other tablet shaped candies, or the like), noodles (such as Japanese buckwheat noodles called "Soba", Japanese wheat noodles called "Udon", Chinese noodle or the like), dairy products (such as milk, ice cream, yogurt or the like), seasoning (such as fermented soybean paste called "Miso", soy sauce, or the like), soups and drinks (such as juice, soft drinks including carbonated drinks, coffee, black tea, green tea, isotonic drinks,

or the like), of healthy food (such as food in supplement in tablet, capsule or the like), nutraceutical supplement (such as nutrition supplement drinks or the like). Extract (polyphenol) derived from evening primrose seed is accordingly applied to those above or convenience food. For example, ethanol extract of evening primrose seed mixed with powdered cellulose can be spray-dried or freeze-dried and converted into powder, granule, tablet, solution or the like, and then applied to various food and drink.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 on the attached sheet is a graph indicating a variation of blood glucose level of a normal rat after extracts of evening primrose seed and starch have been administered to the said rat.

Fig. 2 on the attached sheet is a graph indicating a variation of blood glucose level of a normal rat after extracts of evening primrose and sucrose have been administered to the said rat.

(Additional part)

Fig. 3 on the attached sheet is an HPLC chart of the extract in this invention

Fig. 4 on the attached sheet is a graph indicating a suppressive effect on the elevation of blood glucose level of the nondiabetic subject.

Fig. 5 on the attached sheet is a graph indicating a suppressive effect on the elevation of blood glucose level of the mild diabetic subjects.

(End of the additional part)

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Embodiments of this invention are as follows.

(Manufacture of the carbohydrate absorption inhibitor)

Material: Compressed cake obtained in the manufacturing process of evening primrose oil is used.

Firstly, the compressed cake was crushed, refluxed by hexane, and then the defatted substance was obtained by removing the remaining oil from the said compressed cake. Secondly, the said defatted substance was fluxed in 70 % (v/v) aqueous ethanol, and then the said ethanol extract was evaporated until carbohydrate absorption inhibitor (Embodiment 1) was obtained.

(Inhibitory activity against α-amylase)

Inhibitory activity (5) of α-amylase in regard to the Embodiment 1 (extract of evening primrose seed) was determined by using an amylase activity measuring kit ("Amylase Test Wako" manufactured by Wako Pure Chemical Industries, Ltd., Japan) , which is commercially available. Measuring method using human saliva-derived α-amylase is as follows.

After incubating a mixture of the human saliva-derived α-amylase solution (0.02mL) and sample solution (0.01mL) at 37℃ for 5 min., substrate buffer solution (1.0mL) was added to the said mixture and incubated at 37℃ for 60 min. Then the said mixture was incubated in the boiling water for 5 min., and after the reaction stopped, coloring reagent (1.0mL) was added and then the absorbance (660nm) was measured.

After calculating the enzyme activity (%) based on this measured value, the inhibitory activity (5) was calculated by the following formula. The enzyme activity was calculated based on the absorbance (100%) measured under condition where no sample coexisted.

Inhibitory activity (%) = 100 − enzyme activity (%)

Inhibitory activities (%) in regard to other plant-derived extracts of guava leaf (Comparison 1), banaba leaf (Comparison 2) and mulberry leaf (Comparison 3) were also calculated by the same method as shown in the following chart 1.

(Chart 1)

Classification	Extract	Concentration (mg/ml)	Inhibitory activity (%)
Embodiment 1	Evening primrose seed	1.0	100
Comparison 1	Guava leaf	1.0	0
Comparison 2	Banaba leaf	1.0	80
Comparison 3	Mulberry leaf	1.0	0

As described in the above chart 1, it is clear that α-amylase inhibitory activity against the embodiment 1 is higher than any of the others.

Also, after measuring the enzyme activity to the variation of concentration regarding the embodiment 1 (evening primrose seed extract) by using the same method, IC_{50} (50% inhibition concentration) against the α-amylase (derived from human saliva) was found to be 0.5mg/mL.

In connection with the embodiment 1 (evening primrose seed extract), gallic acid, ellagic acid, catechin, and pentagalloylglucose, IC_{50} (50% inhibition concentration) against the α-amylase derived from pancreas of pig was

identified by the same method. The following chart shows the excellent inhibitory activities.

Chemical compound	IC_{50} (mg/ml)
Embodiment 1 (evening primrose seed extract)	0.01
Gallic acid	0.4
Ellagic acid	> 1.0
Catechin	0.8
Pentagalloylglucose	0.02

[α-glucosidase inhibitory activity]

Next, α-glucosidase inhibitory activity (%) in regard to the embodiment 1 (evening primrose seed extract) was identified by the following method. As for the α-glucosidase for this measurement, 0.1 mol maleic acid buffer solution (pH6.0) was added to the acetone powder in the intestinal canal of rat (SIGMA CHEMICAL CO.) and homogenized in ice by using a glass homogenizer, and then centrifugalized supernatant was diluted 20 times. Measuring method is shown below.

Sample solution (0.01ml) was added to the enzyme solution (0.05ml), then 0.1 M maleic buffer solution (0.64 ml), moreover, 33 mM 4-methylumbelliferyl-α-D-glucoside (0.3ml) was added and then incubated at 37℃ for 30 min.

After that, the reaction was suspended by adding 3 ml glycin buffer solution (pH10.3) and the quantity of the isolated 4-methylumbelliferyl was determined by using a fluorometer (excitation wavelength: 366nm & fluorescent

wavelength: 450mm). Enzyme activity was calculated based on this determined value and inhibitory activity (%) was calculated by the following formula. Also, the enzyme activity was calculated based on the determined value (100%) of 4-methylumbelliferyl measured under condition where no sample coexisted.

Inhibitory activity (%) = 100 − Enzyme activity (%)

As a comparison, the inhibitory activities (%) in regard to the plant-derived extracts – guava leaf extract (Comparison 1), banaba leaf extract (Comparison 2), and mulberry leaf (Comparison 3) were identified by the same method. Result is shown in the following chart 2.

(Chart 2)

Classification	Extract	Concentration (mg/ml)	Inhibitory activity (%)
Embodiment 1	Evening primrose seed	0.1	65
Comparison 1	Guava leaf	0.1	77
Comparison 2	Banaba leaf	0.1	76
Comparison 3	Mulberry leaf	0.1	96

As indicated in Chart 2, although inhibitory activity against α-glucosidase of embodiment 1 (evening primrose seed extract) is less effective than that of guava leaf extract (Comparison 1), banaba leaf extract (Comparison 2) and

mulberry leaf extract (Comparison 3), it still has excellent inhibitory activity against α-glucosidase.

After measuring the enzyme activities to the variation of concentration in regard to the embodiment 1 (evening primrose seed extract), gallic acid, ellagic acid, catechin and penthagalloylglucose by using the same method, IC_{50} (50% inhibitory concentration) against α-glucosidase was identified.

The following chart shows the excellent inhibitory activities.

Chemical compound	IC_{50} (mg/ml)
Embodiment 1 (evening primrose seed extract)	0.1
Gallic acid	0.8
Ellagic acid	> 0.08
Catechin	0.8
Pentagalloylglucose	0.04

Those test results indicate that the embodiment 1 (evening primrose seed extract) comprises the inhibitory activity against both α-amylase and α-glucosidase, and effectively inhibits the degradation of carbohydrate.

(Carbohydrate loading test to normal rats)

In regard to the embodiment 1 (evening primrose seed extract), carbohydrate absorption inhibitory action in living animals was tested under the following conditions.

After breeding 5-week old male wister rats (Clear Japan, Inc.) for more than one week, then 6 to 8-week old wister rats were used during the test. Without giving any food to the rats for 24 hours, sample of Embodiment 1(at the rate of 0.5g per kg of weight) was dissolved in distilled water, and carbohydrate (starch and sucrose: at the rate of 2 g per kg of weight) was dissolved in distilled water, then both were administered to the stomach sound.

To measure a variation of the blood glucose level after administering the samples to the rats, blood was taken from the vain of tails before the samples were administered, and at each stage of 30, 60, 120 and 180 min after the samples were administered. As a control group, blood was taken from the vain of the tails of the rats under the same conditions where the sample, the same amount of distilled water and carbohydrate (starch and sucrose at the rate of 2 g per kg of weight) were administered to the rats.

After centrifuging the blood taken from the rats, the blood glucose level of the supernatant was measured. For measuring the blood glucose level, a glucose measuring kit commercially available was used. ("Glucose B Test Wako" manufactured by Wako Pure Chemical Industries, Ltd., Japan) The results are shown in Fig. 1 and Fig. 2.

As shown in Fig. 1 and Fig. 2, in a control group, the blood glucose level rapidly increased before 30 min passed, on the contrary, the blood glucose level of the rats of which the embodiment 1 (evening primrose seed extract) was administered slowly increased.

(Application to food and drink)

Application to food and drink which are effective for the prevention of diabetes mellitus and diet are as follows. "Evening primrose seed extract" shown below is powdered and dried ethanol extract of evening primrose seed obtained under the same conditions of the aforementioned embodiment 1.

Composition 1: Chewing gums

Sugar	53.0wt%
Gum base	20.0
Glucose	10.0
Starch syrup	16.0
Flavoring agent	0.5
Evening primrose seed extract	0.5
	100.0wt%

Composition 2: Gummy candies

Reduced syrup	40.0wt%
Granulated sugar	20.0
Glucose	20.0
Gelatin	4.7
Water	9.68
Japanese apricot (Ume) juice	4.0
Japanese apricot (Ume) flavor	0.6
Coloring agent	0.02
Evening primrose seed extract	1.0
	100.0wt%

Composition 3: Candies

Sugar	50.0wt%
Starch syrup	33.0
Water	14.4
Organic acid	2.0
Flavoring agent	0.2
Evening primrose seed extract	0.4
	100.0wt%

Composition 4: Soft capsule

Rice germ oil	87.0wt%
Emulsifying agent	12.0
Evening primrose seed extract	1.0
	100.0wt%

Composition 5: Soft drink

Fructose and glucose	30.0wt%
Emulsifying agent	0.5
Evening primrose seed extract	0.05
Flavoring agent	Minute amount
Distilled water	Rest
	100.0wt%

Composition 6: Tablets

Lactose	54.0wt%

Crystallized cellulose	30.0
Starch degradation product	10.0
Glycerin fatty acid ester	5.0
Evening primrose seed extract	1.0
	100.0wt%

Composition 7: Tablet-shaped candies

Sugar	76.4wt%
Glucose	19.0
Glycerin fatty acid ester	0.2
Evening primrose seed extract	0.6
Distilled water	3.9
	100.0wt%

The structure of the extract obtained from the embodiment 1 and similar method contained ellagic acid, gallic acid, catechin, and pentagalloylglucose.

(Beginning of the additional part)

Analysis in regard to evening primrose seed extract in this invention is shown in chart 3 below.

Chart 3

Sample	Gallic acid	Catechin	Ellagic acid	PGG*1
Evening primrose extract Lot: R-006	2.7%	1.6%	0.5%	2.3%

*1: Pentagalloylglucose

Also, HPLC chart is shown in Fig.3. Considering the above result, it is recognized that the extract obtained in this invention contains gallic acid, catechin, ellagic acid and pentagalloylglucose. It is still impossible to create a sample excluding the specific component from the evening primrose seed extract even by the present technology. Therefore, performance test should be still conducted by using a mixture of various components. After measuring the effectiveness to inhibit the after-meal elevated blood glucose level of 16 normal persons (female : male = 7 : 9), the result in Fig. 4 shows that blood glucose level of subjects in 30 minutes after taking evening primrose is controlled. Also, the rise in blood glucose level of the patient suffering from the mild diabetes mellitus with the fasting blood glucose level of 110mg/dl to 180mg/dl is more significantly controlled than the rise in postprandial blood glucose level of the patient in a placebo group.

Chart 4 :Change in the value of insulin when conducting the food loading test (μ U/dl)

	Before loading test	30 min	60 min	90 min	120 min
Control group	13.3±15.0	28.4±23.9	40.0±31.1	43.6±28.1	50.1±30.0
Evening primrose extract group	12.7±9.8	22.4±14.5	35.8±33.1	43.7±35.9	43.7±31.4
Significant difference between groups	n.s	n.s	n.s	n.s	n.s

It is apparent that evening primrose comprises an effective function to control the rapid rise in blood glucose level after food loading. Since the rapid rise in blood glucose level is controlled, the rapid increase in insulin secretion becomes moderate. This indicates that evening primrose extract is effective to

reduce the burden of insulin secretion of the patient suffering from insulin deficiency diabetes mellitus.

(End of the additional part)

Industrial applicability

(Beginning of the additional part)

The amount of carbohydrate taken into the human body can be controlled by inhibiting the digestion of starch and saccharides, i.e., by inhibiting the activity of α-amylase and α-glucosidase which are saccharides degrading enzyme inherent in living body. The risk of type II diabetes mellitus can be especially controlled by a mechanism which keeps the postprandial blood glucose level low.

The said mechanism is significantly safer and harmless than the direct prevention to control the insulin secretion, and from the viewpoint of the preventive medicine in the latest mainstream of alternative medicine, it is considered to be inventive that the said mechanism can be applied to the function of the evening primrose leaf extract.

Also clinical investigation of the diabetes patient apparently shows that administration of evening primrose does not directly enhance the insulin secretion, and decrease in the blood glucose level of human and animals apparently indicates that evening primrose extract inhibit the absorption of the saccharides.

(End of the additional part)